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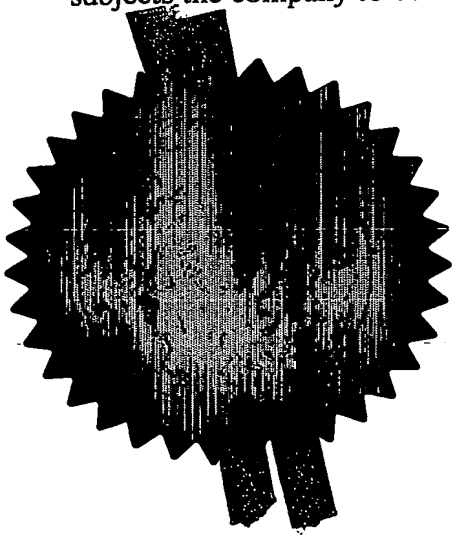

Signed R. Mahoney

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P01/7700 0.00-0324482.9

Request for grant of a patent

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21 OCT 2003

The Patent Office
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1. Your reference

P/242/GBA

2. Patent application number
(The Patent Office will fill in this part)

0324482.9

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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&

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Patents ADP number *(if you know it)*

8736923001 U.K

If the applicant is a corporate body, give the country/state of its incorporation

Singapore
8737017001

4. Title of the invention

Methods for the treatment of cancer

5. Name of your agent *(if you have one)*

Jacqueline G V Evans et al.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Greaves Brewster
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Patents ADP number *(if you know it)*

8859080002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Methods for the treatment of cancer

Field of the invention

The present invention relates to methods for the treatment of cancer by chemo-brachytherapy. In particular, the invention relates to methods for the treatment of cancer by chemo-brachytherapy using pharmaceutical compositions comprising chlorambucil and a porous carrier material.

Background to the invention

The principal methods of treatment of cancer in common use include surgery, chemotherapy, and radiation therapy. These therapies all have serious limitations associated with their use.

Surgical resection, for example, is limited by the ability to expose and remove the tumour, and is ineffective against micro-metastases that may have migrated from the site of the primary tumour.

The effectiveness of radio- and chemotherapy is limited by the ability to target the tumour without damaging healthy tissue. In the case of chemotherapy, for example, poor specificity of the drugs for cancer cells typically results in systemic toxicity before suitable therapeutic drug levels in the tumour can be achieved. Further, as chemotherapeutic drugs usually act on rapidly dividing cells, the cells of the intestinal lining and bone marrow can be extensively damaged during the treatment. Additionally, the effectiveness of chemotherapy is often hampered by drug resistance.

Radiation therapy can be specifically directed to the site of the tumour, but is also limited by the potential damage to non-cancerous tissue. Radiation therapy relies on the ability of imaging techniques to identify tumour sites for treatment. If the tumour is too

fluoroacil, vinblastine, GNRH and cis-platin. It is suggested that implants comprising an anti-cancer component selected from a radionuclide and/or a cytotoxic drug and a silicon component may be suitable for brachytherapy.

As yet, no effective localised chemotherapy products based on silicon as a delivery vehicle are commercially available, however, and there remains continuing interest in developing further and improved methods for localised chemo-brachytherapy of cancer tumours in order to extend the range of treatment options available to the physician.

Summary of the invention

The present invention is based on the finding that the effectiveness of the known anti-cancer agent chlorambucil in tumour regression can be improved markedly when it is formulated for local delivery by controlled release from a porous carrier material.

Chlorambucil (chemical name 4-[bis(2-chloroethyl)amino}benzenebutanoic acid) is a cytotoxic, alkylating agent which is well known for use in cancer therapy. It is used as an antineoplasic agent to treat chronic lymphatic leukemia, malignant lymphomas, giant follicular lymphoma and Hodgkin's disease and is generally available in tablet form for oral administration.

The present inventors have found that delivery of free chlorambucil 'locally' into tumours has limited success in restricting tumour growth and moreover that this leads to the rapid systemic distribution of the drug, resulting in systemic toxicity. Surprisingly, however, the present inventors have found that chlorambucil when impregnated in a porous carrier system for local delivery is highly effective in effecting tumour regression, particularly when the porous carrier system carries a high loading of chlorambucil for release, in active form, at the location of the cancer.

The use of the porous carrier enables improved localization of the drug in the tumour, thereby minimizing systemic toxicity, and also providing for far higher tumour-

invention affords the possibility of achieving controlled release of the drug, leading to slow treatment of the tumour over a period of time. Slow regression is advantageous as the build up of necrotic tissue is reduced.

Detailed description of the invention

By 'chemo-brachytherapy' is meant a method of treatment of cancer by localized chemotherapy in which a cytotoxic drug is introduced in or near to the tumour itself.

As used herein, a 'porous carrier material' is any porous material which is capable of acting as a carrier for chlorambucil. The chlorambucil may be deposited on the surface of the porous carrier material or may be bound or otherwise associated with the surface of the material but preferably the chlorambucil is incorporated into the pores of the porous carrier material.

Preferably the porous carrier material for use according to the invention is capable of being loaded with high levels of chlorambucil distributed with high uniformity throughout substantially the entire porous material for release, in active form, at the intended site of action. Porous carrier materials which allow for release of high levels of chlorambucil, in active form, at the intended site of action and in a controlled manner over a period of time are particularly preferred.

The porous carrier material may suitably comprise a semiconductor, such as doped or undoped silicon carbide, silicon nitride or germanium. Preferably the porous carrier material comprises the semiconductor silicon.

Porous silicon may be classified depending upon the nature of the porosity (the porosity is the fractional void content by volume). Microporous silicon has an average pore size of less than 2 nm, mesoporous silicon has an average pore size between 2 and 50 nm and macroporous silicon contains pores having a diameter greater than 50 nm. Certain forms of porous silicon have been found to be be resorbable, as described in WO 97/06101.

doping (with resistivities in the order of 0.005 ohm.cm^{-1}). In the present invention, the silicon used is preferably derived from p+ silicon.

The porous silicon may comprise a single sample of porous silicon (in which any part of the porous silicon is substantially integral with the remaining parts of the sample), suitably of thickness between 100nm and 1 mm, preferably between 1 micron and 750 microns. Alternatively, and preferably, the porous silicon may be in particulate form. Preferably the composition for use according to the invention comprises a multiplicity of porous silicon microparticles having a mean particle size of between 100 nm to 10 microns, preferably 500 nm to 2 microns. The multiplicity of porous particles preferably comprises a multiplicity of particles having the same shape and more preferably also the same volume as each other. The particles are each preferably substantially symmetrical and may be substantially oval, spherical or in the form of microneedles.

Porous silicon particles for use in the invention may be prepared by a number of known techniques. For example, a single crystal wafer silicon may be porosified by anodisation, for example, using HF and an electric potential. Alternatively, microparticles derived from polycrystalline feed stock may be manufactured by a two stage process, firstly by jet-milling the particle size from a few millimeters to a uniform micron sized stock followed by stain etching through established methods.

The present inventors have found that the use of compositions wherein the chlorambucil is present at a high loading level is preferred.

The compositions for use according to the invention preferably contain at least 15 % by weight of cytotoxic drug, based on the weight of the composition. Preferably the drug is present in an amount of from 15% to 85% by weight, particularly from 20% to 50% by weight, especially from 30% to 45% by weight, based on the weight of the composition.

It will be appreciated that these high loading levels by weight equate to a high percentage by volume of the pores in the porous carrier material being occupied by the drug. The

occupied by the chlorambucil) is preferably in the range of from 30% to 100%, especially from 50% to 90%.

Compositions for use according to the method of the invention having high loading levels of cytotoxic drug , such as chlorambucil, distributed with high uniformity throughout substantially the entire porous material may suitably be prepared by a method comprising the steps of:-

i) bringing the cytotoxic drug into contact with the porous carrier material; and
ii) allowing the cytotoxic drug to impregnate the porous carrier material, the impregnation being performed at a temperature which is at or above the melting point of the cytotoxic drug.

This may be achieved by:-

i)heating the porous carrier material to a temperature at or above the melting point of cytotoxic drug;

ii) bringing the cytotoxic drug into contact with the heated porous carrier material,; and

iii)allowing the cytotoxic drug to impregnate the porous carrier material.

Alternatively, the impregnation may be brought about by the steps of:-

i) heating the cytotoxic drug to a temperature at or above its melting point ;

ii) bringing the molten cytotoxic drug into contact with the porous carrier material ; and

iii)allowing the cytotoxic drug to impregnate the porous semiconductor.

In a yet further embodiment, both the porous carrier material and cytotoxic drug may be heated independently to a temperature at or above the melting point of the drug and then brought into contact together to allow impregnation to occur.

appropriate organ may be surgically debulked and the residual space filled with composition or the organ may be cored with an array of needles and the cores filled with the composition, for example.

Preferably, the composition may be formulated for parenteral administration to be given as an injection (for example, intravenous, intravascular, subcutaneous, intramuscular or by infusion). The composition for injection may conveniently take the form of a suspension. The present inventors have found that excipients based on long and medium chain triglyercides, or derivatives of these, such as arachis oil, sesame oil and Captex® 355 (triglycerides of caprylic/capric acid) and Cremopher EL are particularly useful in parenteral formulations, as are excipients which have surfactant properties such as polysorbates.

It will be appreciated that the porous material may be formed into an implantable implant or made into particulate form either prior to or after loading with the drug.

Preferably, the compositions for use according to the invention comprise a multiplicity of microparticles, each microparticle comprising the drug and a porous carrier material.

The composition may conveniently be introduced to the site at which the cancer is located by injecting a suspension of microparticles into an artery or vein connected to or located in the organ(s) in which the tumour is located. This provides for an even distribution of drug throughout the affected area.

Alternatively, the method according to the invention may comprise direct intratumoural introduction of the composition. Compositions in the form of microparticles may be delivered using a fine bore needle and this represents a significant advantage in terms of ease of administration.

In order accurately to guide the introduction of the pharmaceutical composition according to the method of the invention, the tumour may be imaged using a number of known

Figure 5 shows the dose-dependent regression of human tumour in mice following intratumoural injection of chlorambucil delivered by porous silicon. (CBS). The control group received intratumour injection of 100ul of peanut oil only.

Figure 6 shows the acute toxicity of chlorambucil 720ug (LD-50), 1500ug (LD-100) as free drug and as incorporated into porous silicon (CBS).

Example 1

Preparation of porous silicon microparticles.

Porous silicon microparticles were prepared from either single crystal wafer silicon (purity 99.99999%) or from polycrystalline feed stock material (purity 99.999%). In the first case, silicon surfaces were porosified in a double-tank anodisation cell. Samples of P-type boron doped silicon wafer were anodised in a solution of HF : EtOH. The samples were rinsed with ethanol and dried by using a spin dryer. The resulting pore size distribution was monitored by high resolution SEM microscopy (JEOL 6400F) and the thickness of the porous layer was measured using cross sectional SEM.

Microparticles derived from polycrystalline feed stock were manufactured by jet-milling the particle size from a few millimeters to a uniform micron sized stock followed by stain etching using conventional methods. Porous silicon microparticles derived from stain etching were poly-Si mm spheroids jet milled to 4 micron diameter and then reacted in a staining solutions containing water / HF/ Nitric acid for typically 3-30 minutes.This generated powder with a typical surface area of 50-100m2/g.

Weight of unloaded flake = 44.34 mg

Density = d_{chl} g cm^{-3}

Void volume = {44.34/ 479.60 mg} x 814.75 mg/ {density of silicon}
= 0.03233 cm^3

Maximum loading capacity = 0.03233 cm^3 x d_{chl} g cm^{-3}
= 32.33 d_{chl} mg

Loading & washing w_1 = 71.66 mg
w_2 = 73.41 mg
w_3 = 72.92 mg equivalent to 39.19 % w/w

% loading capacity (w/v) = {72.92 – 44.34}/ {32.33 d_{chl}} x 100
= 88.40/ d_{chl} %

Compositional analysis of the impregnated membrane, using the SEM-EDX method (Figure 1), revealed that the loading of chlorambucil is fairly homogenous through the membrane, with reference to C/Si and Cl/Si ratios. High chlorambucil is detected, as evidenced with C/Si ~ 0.35 on average.

Following incorporation into the membrane, chlorambucil was extracted and subjected to HPLC analysis. A known amount of the incorporated Chlorambucil/porous silicon (typically 10-15mg, dependent on the level of incorporation) was placed into a volumetric flask and made up to a volume with 100ml of ethanol, shaken and sonicated for 30 minutes at 30°C. From this stock solution 25ml were transferred by pipette to a 100ml volumetric flask to give the sample solution. Prior to analysis by HPLC the sample was filter through a 0.45micron filter.

Cells were cultured according to the following protocol:-

1. Cells are seeded in 1ml media/well in 24 well plates at the appropriate density (pre-determined e.g. for Hela's = 40,000/well) so that cell confluency is 60 – 70% at the start of the experiment.
2. Plates are then left overnight at 37 °C, 5% CO_2 to adhere.
3. Trans-well inserts are added to each well of the 24 well plates prior to preparation of the formulations.
4. Samples of porous silicon impregnated with chlorambucil were weighed out and suspended in Mc Coy's 5a medium, supplemented with 10% fetal calf serum (FCS), 2mM Glutamine. Controls were set up with medium containing no chlorambucil and with medium containing free chlorambucil, solubilised in ethanol. 100µL of each formulation was added to each appropriate trans-well insert in triplicate and returned to the incubator for 24hrs prior to analysis of viability.

The Neutral Red assay used is described by Triglia, D., et al, In Vitro Cell. Dev. Biol. **27A**, 239 (1991) and Fautz, R., et al., Mutat. Res. **253**, 173-179 (1991).The following procedure is followed:-

1. A 4mg/ml neutral red stock in water is used to prepare a 50µg/ml working solution in pre-warmed culture media, allowing 0.5ml per well for treatment.
2. Inserts & culture media are removed from each well, cells gently washed with PBS and neutral red solution added followed by incubation at 37 °C for 45 mins.
3. Dye is then removed, cells re-washed with PBS x 2 to remove unincorporated dye & 0.5ml destain solution (50% water, 49% Ethanol, 1% Glacial acetic acid) added to each well to solublise all incorporated dye.
4. 90µL of each sample is then aliquoted in triplicate into a 96 well plate (including destain control) & absorbance of the plate at read at 540nm
5. Neutral Red uptake of the treated wells is expressed relative to the untreated ones to give relative cell viability.

2. Animals groups: Animals were randomly grouped into control control 1 (injected with peanut oil), control 2 (injected with porous silicon without drug), free chlorambucil (drug injected without porous silicon directly into tumour or injected via intraperitoneal route) and chlorambucil impregnated porous silicon groups. Each group included 16 to 20 animals.

3. Injection of chlorambucil impregnated porous silicon in transplanted tumours: On day 14 after implantation of tumours (diameter of the tumour about 1cm), the chlorambucil impregnated formulation was applied to the centre of the tumours.

4. Tumour volumetrics: The sizes of implanted tumours in nude mice was estimated every 3 days. The largest and smallest diameters were measured by a vernier caliper and tumour volumes estimated according the formula: $V=1/2\ ab^2$, where a and b are largest and smallest tumour diameters respectively, and V is the tumour volume in cm^3.

The effects of intra-tumoural administered chlorambucil loaded porous silicon on the growth of the tumour was studied and compared with control and systemic therapy groups .The body weight of the animals was estimated by subtracting the tumour volume (cm3) from the total body weight (g) every 3 days. The survival time of each experimental animal was recorded.

As can be seen from the results of the relative tumour volumes over time presented graphicially in Figure 4, chlorambucil loaded into porous silicon (CBS) was very effective in causing tumour regression depending on the dosage used. These results are from anodisation derived porous silicon flake loaded with chlorambucil (360ug or 720ug) by the melting solvent method and subsequently subjected to particle size reduction to about 20 microns by grinding in a pestle and mortar for 1 hour

In another series of experiments using the same tumour model, higher dosage of chlorambucil (1500ug), delivered by porous silicon and injected directly into the tumour,

Claims

1. A method of treating a cancer by chemo-brachytherapy, the method comprising introducing to the site at which the cancer is located a pharmaceutical composition comprising chlorambucil and a porous carrier material.

2. A method according to claim 1 wherein the porous carrier material is doped or undoped silicon, germanium, silicon carbide or silicon nitride

3. A method according to claim 2 wherein the porous carrier material is silicon

4. A method according to claim 3 wherein the silicon is resorbable

5. A method according to claim 4 where the silicon is mesoporous

6. A method according to any of claims 3 to 5 wherein the porous silicon has a porosity of from 40% to 80%

7. A method according to any preceding claim wherein chlorambucil is present in the pharmaceutical composition in an amount of from 15% to 85% by weight, based on the weight of the pharmaceutical composition.

8. A method according to claim 7 wherein chlorambucil is present in the pharmaceutical composition in an amount of from 30% to 45% by weight, based on the weight of the pharmaceutical composition.

9. A method according to any preceding claim wherein the pharmaceutical composition comprises a multiplicity of microparticles

10. A method according to claim 9 wherein the pharmaceutical composition is introduced to the site at which the cancer is located by injecting a suspension of microparticles into an artery or vein connected to or located in the organ(s) in which the cancer tumour is located.

Abstract

The invention provides the use of chlorambucil in chemo-brachytherapy and a method of treating a cancer by chemo-brachytherapy using chlorambucil, the method comprising introducing to the site at which the cancer is located a pharmaceutical composition comprising chlorambucil and a porous carrier material. Also provided is the use of a porous carrier impregnated with a cytotoxic drug, such as chlorambucil, to deliver the drug at a dose higher than the LD50 for the free drug.

Figure 1

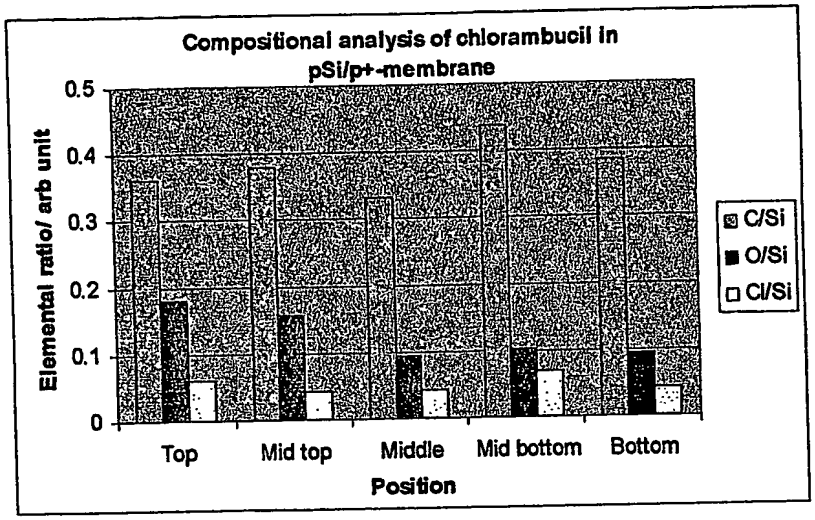
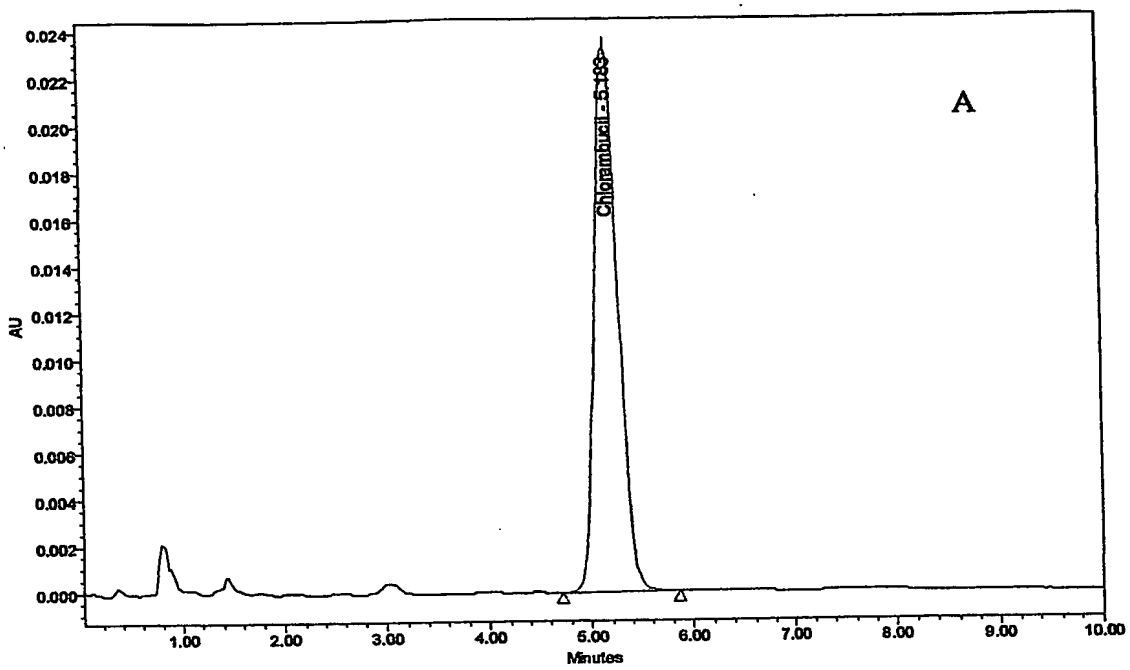

Compositional analysis of chlorambucil in pSi/p+-membrane
Elemental ratio/ arb unit
0.5
0.4
0.3
0.2
0.1
0
Top
Mid top
Middle
Mid bottom
Bottom
Position
C/Si
O/Si
Cl/Si

Figure 2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0.020
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0.016
0.014
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0.008
0.006
0.004
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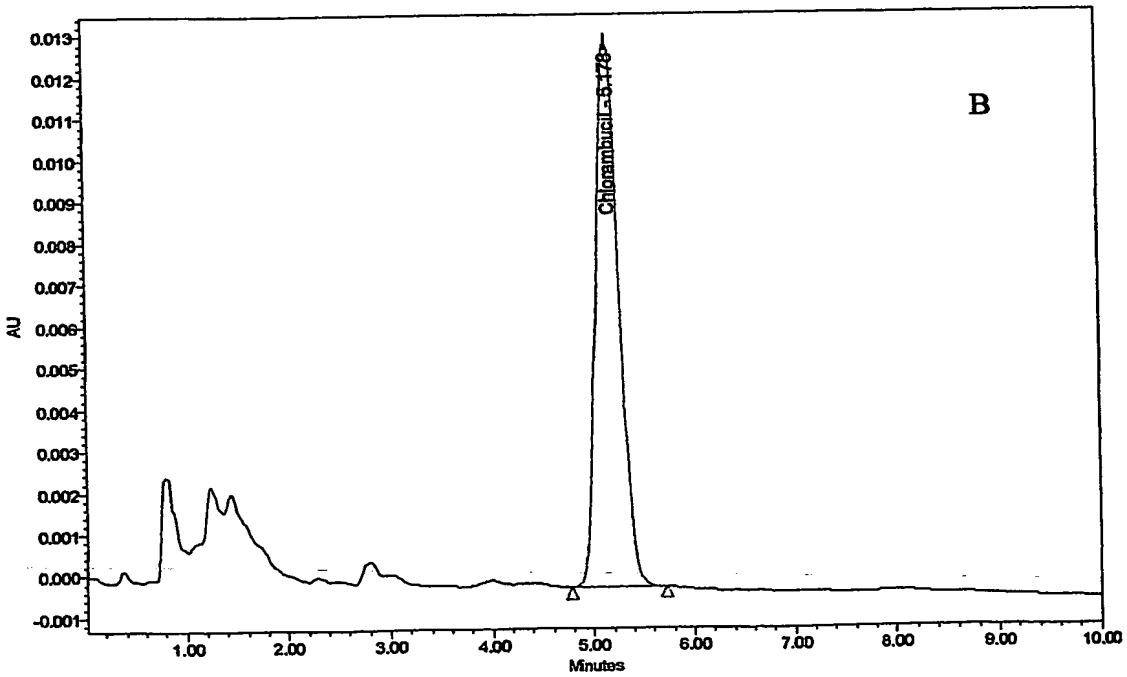
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AU
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Figure 3

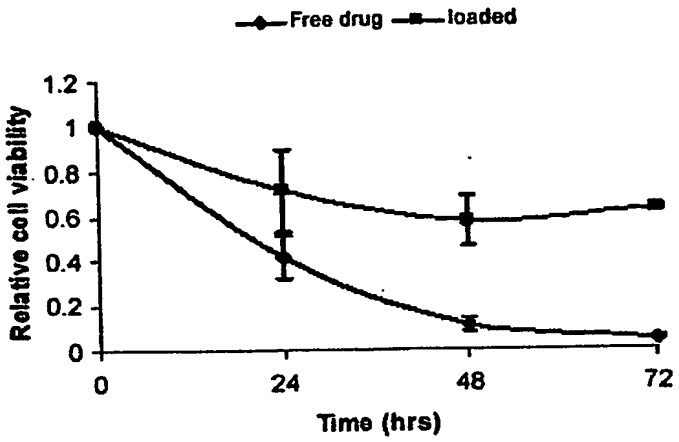

Figure 4

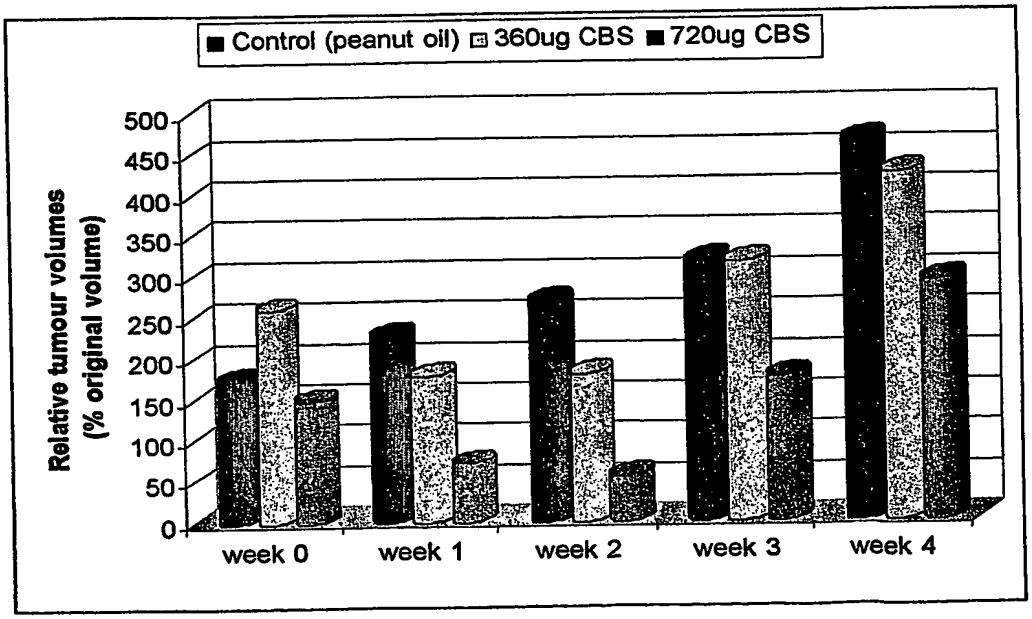

Figure 5

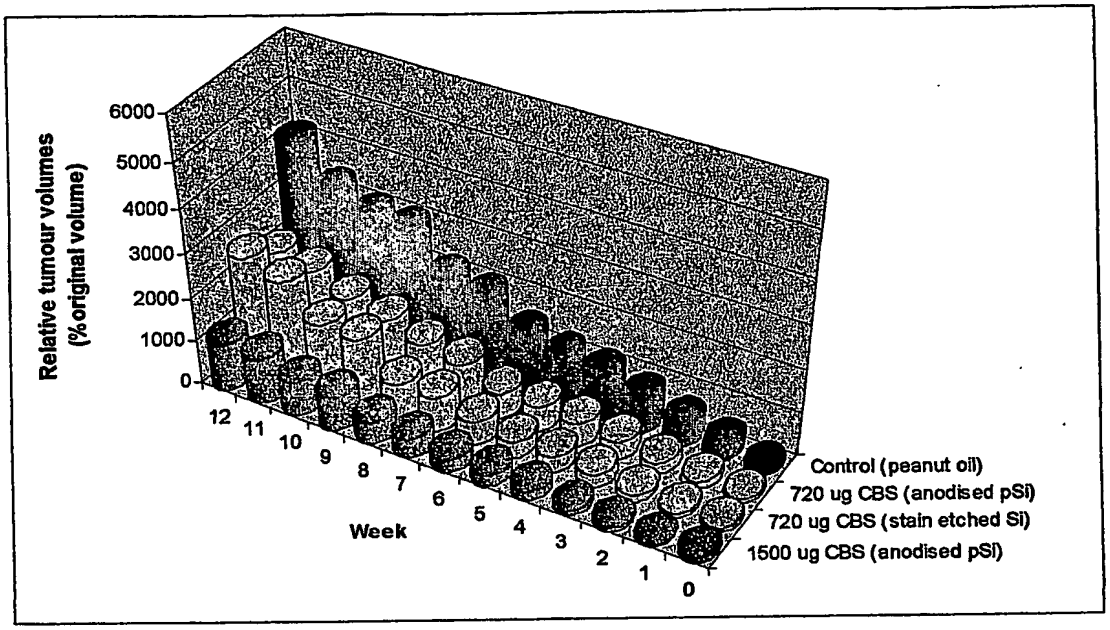

Figure 6